**Case 26: Chronic Granulomatous Disease (CGD)**

**Summary:** Macrophages and neutrophils destroy invading microbes in both innate and adaptive immune responses using phagocytosis. Uptake is enhanced by opsonization of the particle with complement (or antibody and complement in the adaptive response). This involves the production of hydrofren peroxide and superoxide radicals results in changes to phagosomal pH and membrane potential, as well as production of bactericidal factors and enzymes that are released into the phagosome.

NADPH oxidase is an enzyme complex that catalyzes the initial reaction to generate superoxide radicals using oxygen. The superoxide is then dismutated in the presence of H to produce hydrogen peroxide and oxygen. The NADPH oxidase complex is a large multisubunit enzyme complex whose assembly in the phagosome membrane is triggered by phagocytic stimulus. Cytochrome b558 complex contains the catalytic site and consits of the heavy gp91 chain and light p21 chain in the granule membranes. Other subunits (p47, p67, p40, Rac2) translaocate to the membrane to interact with cytochrome B to form the active NADPH oxidase that shuttles electrons from cytosolic NADPH to molecular oxygen.

P47, p67, p21 are encoded on autosomal chromosomes while gp91 is encoded on the short arm of the X chromosome. X linked CGD is the most common form and is caused by mutations in gp91.

Males affected commonly show susceptibility to infection during the first year of life including pneumonia, infection of lymph nodes (lymphadenitis) and abscesses of skin and viscera (liver). Granuloma formation occurs when intracellular microbes are opsonized and injested by phagocytes but the infections can’t be cleared and the infected macrophages accumulate. Persistent presentation of microbe antigens by phagocytes induces a sustained cell mediated response via Th1 CD4 T cells, which recruit other inflammatory cells and set up chronic local inflammation because of sustained activation of chronically infected phagocytes that can’t destroy the bacteria that they ingest (granuloma). In CGD patients, the intracellular microbicidal mechanisms are defective so infections persists and chronic inflammatory reactions result in granuolma formation. Pathogens include mycobacterium tuberculosis. The core of the granuloma consistes of infected macrophages and may include multinucleated giant cells consisting of fused macrophages surrounded by large macrophages (epithelioid cells). These can be surrounded by CD4 T cells.

If a patient also has an issue with chemotaxis there is probably a defect in the function of Rac2 which is used in the function of membrane cytochrome b complex and for chemotaxis of neutrophils.

Diagnosis of CGD is made by using the nitro blue tetrazoium (NBT) dye, which is pale yellow and transparent. When it is reduced it becomes insoluble and deep purple. A drop of blood is suspended on a slide and treated with a phagocytic stimulus to activate the oxidase (ex: 12 myristate 13 acetate PMA) and NBT is added. Neutrophils in the blood take up NBT and the PMA. Normal result is NBT reduction to deep purple with insoulble formazan seen in phagocytic cells. IN CGD blood no dye is reduced. The dye hyhydrorhodamine (DHR) can also be used. Whole blood is stained with DHR and the blood is stimulated with PMA. Normal phagocytes will produce superoxide radicals that reduce DHR to highly fluorescent molecule rhodamine that can be measured with fluorescence activated cell sorting (FASC). CDG patient cells will fail to reduce the DHR and will remain non-fluorescent.

Monoclonal Abs against CD18 can induce a mild phenotype of LAD, used to prevent graft rejection in kidney graft recipients and when administered before bone marrow transplants they can prevent graft vs. host disease

**Case:** 15 yr old boy with a gardening job, rapidly developed SOB, persistent cough, and chest pain. CXR showed large cotton ball densities in both lungs, aspirates showed fungal hyphae and aspirate grow aspergillus fumigatus (treated with IV amphotericin B and ventilation), 2 other respiratory infections with pseudomonas aeruginosa and strep faecalis, normal WBC count, serum IgG, IgM and IgA are high normal (because of persistent antigenic stimulation so he makes more Abs than normal, all chronic infections result in hypergammaglobulinemia such as malaria), NBT test showed that WBCs couldn’t reduce the dye, brother also couldn’t reduce this (also had perirectal abscess in infancy), mom and sister had mixed population of neutrophils (half reduced NBT and half didn’t), low hydrogen peroxide production and cytochrome B content was low, treated with IFNg

**Treatment:** IFN g improves resistance, severe cases may warrant bone marrow transplants and is the only currative treatment by replacing defective blood cell progenitors of the patients with normal progenitors from the donor (carries risks and complications and success depends on availability of HLA matched donor), gene therapy has been attempted and is showing promise (progenitor blood cells collected from patients and were infected with virus containing a normal copy of the gene that is mutated in the patient, corrected cells then injected back into the patient to result in development of a population of normal phagocytes that are able to produce superoxides).

**Explain that chronic granulomatous disease (CGD) is found when NADPH oxidase is non-functional due to mutations affecting one of its components.**

**Explain that NADPH oxidase catalyzes the formation of superoxide radicals**

**Describe that in the presence of hydrogen ion, superoxide will undergo dismutation to form hydrogen peroxide and oxygen.**

**Explain that failure of activated neutrophils to reduce nitroblue tetrazolium (NBT) is a marker of CGD**

**Explain that, though the most common form of CGD is X-linked, other forms that are not due to X-linked mutations are known, and these affect different subunits of NADPH oxidase**

**Describe the pattern of NBT reduction shown by neutrophils in normal persons, maternal carriers of X-linked CGD, and in affected boys. In the carriers, explain why neutrophils will show random X inactivation. Describe the X-chromosome inactivation pattern found for only those neutrophils from carriers that reduce NBT.**

Random inactivation of X chromosomes in female carrier neutrophils. Half of her neutrophils have the normal X and half have the X with the CGD defect. Cells with the CDG defective X chromosome haven’t been selected against (so that half won’t reduce NBT but the normal half will).

Affected boys will not reduce NBT and it will remain pale yellow and transparent.

Normal people will reduce NBT and it will turn dark purple and form insoluble formazan.

**Explain that CGD patients are very susceptible to infections by *Staphylococcus aureus*, *Aspergillus*, several Gram-negative rods and actinomycetes, but that they resist infections by streptococci. (This latter is in part due to production of hydrogen peroxide during growth of bacteria and this is not broken down because strep have no catalase)**

Pneumococcus doesn’t produce catalase (converts hydrogen peroxide to water and oxygen) and is less resistant to intracellular killing than catalase producing organisms.

**Case 27: Leukocyte Adhesion Deficiency**

**Summary:** Genetic defect in CD18 cause LAD type 1. Autosomal recessive (chromosome 21). Common beta chain of the 3 beta2 integrins: LFA1 (CD11a:CD18), Mac1 (CD11b:CD18, CR3), and gp150,95 (CD11c:CD18, CR4). Integrins are heterodimeric proteins that contain a beta chain (defines the class) and an alpha chain (defines integrins within a class). LFA1 and Mac1/CR3 bind to Ig superfamily molcecules ICAM1,2, and 3. Mac1 also is a complement receptors and binds iC3b. gp150,95 also binds complement and stimulates phagocytosis. Children with this genetic defect suffer from leukocyte adhesion deficiency. Recurrent bacterial pyogenic infections that are eventually fatal if untreated. If they survive long enough they develop severe inflammation of the gums (gingivitis) because daily neutrophil influx to the oral cavity is important to control bacteria. Problems with wound healing because movement of WBCs into wounds it vital to normal tissue repair (first shown by delayed separation of the umbilical cord and umbilical cord infections). May develop fistuals (abnormal connectin channels) in their intestine after bacterial infections of the gut.

In these patients, encapsulated bacteria are coated as usual with antibody and complement but because neutrophils and monocytes lack the CD19 containing intgrins LFA1 and Mac1/CR3, they are trapped in the bloodstream and can’t emigrate to the site of infection. CR3 can’t bind to opsonized bacteria in neutrophils and CR4 can’t bind to opsonized bacteria in macrophages for uptake.

Kids are unduly susceptible to opportunistic infections (implies normal T cell function despite absence of LFA1 which was thought to be important for T cell adhesion to APCs, T cell binds to APC and intracellular signaling results in conformational change in LFA1 on the T cell that causes it to bind with higher affinity to ICAM3 on the APC). Capacity to form antibodies is also unimparied (shows that adequate collaboration between T cells and B cells can also occur without LFA1). Because neutrophils are released as nromal from the bone marrow into the blood stream, but their migration into tissues is impaired, LAD patients with have remarkable leukocytosis with neutrophilia.

Homing of T cells to lymphoid tissue is normal in patients with LAD and they don’t suffer from susceptibility to opportunistic infections because T cells express beta1 integrin VLA4. Its interaction with VCAM1 on endothelial cells seems to be sufficient to enable T cells to home and function normally. VLA4 isn’t expressed on neutrophils or macrophages, which are more dependent on beta2 integrins for their adhesion. B cells also home normally in LAD patients, probably due to integrin alpha4 beta7 which is fine.

Other causes of LAD: type 2 due to defects in fucose transport in the golgi that impairs selectin mediated leukocyte rolling (impairs transport of fucose into the golgi and prevents fucosylation of newly synthesized glycoproteins such as sialyl Lewis which is the ligand WBCs bind to selectins on the endothelium), type 3 due to mutations in Kindlin3 protein that regulates integrin mediate signaling, Rac2 is a small Rho type GTPase that affections epxression of L selectin, superoxide generation, actin polymerization and chemotaxis (deficiency shows combined features of LAD and chronic granulomatous disease).

LAD differs from chronic granuomatous disease: in LAD there is defect in mobility of leukocytes (emigration from circulation to sites of infection) whereas in CGD the microbicidal capacity of neutrophils and macrophages is impaired. Therefore, kids with LAD never fevelop inflammatory lesions caused by activated phagocytes characteristic of CGD. However, leukocytes in CGD have normal mobility and can reach infection sites normally, but can’t destory the bacteria once they reach them (hence development of abcesses and granulomas). Unlike CGD patients, kids with LAD are no more susceptible to pneumococcal infections than they are to other bacterial infections.

**Case:** 4 wk old girl, swelling and redness around the umbilical cord stumpt (omphalitis) and fever, very high WBC count, cultures from inflamed skin grew e. coli and staph aureus, normal physical exam and chest and ab Xray after she was treated with antibiotics, urine, blood, and CSF cultures were negative but WBC count remained high with normal distrubution of cell types, IgG, IgA, IgM, and complement C3 and C4 were all normal, Rebuck skin window showed that no white cells accumulated on the coverslips although all types of leukocytes were present in abnormally high numbers in her blood, B cells and NK cell levles were elevated, proliferation of T cells in response to PHA was slightly depressed (nonspecific T cell mitogen, response requires cell cell interactions that depend on FLA1 interaction with ICAM as well as VLA4 with VCAM1), flow cytometric analysis showed that her T cells failed to expressed CD18, blood mononuclear cells were stimulated with PHA and examined 3 days later after incubation with a monoclonal antibody against CD11a (alpha chain of LFA1) and none could be detected on the cell surface, had a brother that developed a severe infection of the LI at 2 wks old (necrotizing entercolitis) and separation of his umbilical cord was delayed, he also suffered multiple skin infections and died of staph pneumonia at 1 yr (he also had a very high WBC count)

**Treatment:** bone marrow transplantation with purified CD34 bone marrow stem cells from donor, chemotherapy (before the transplant to destroy recipient abnormal cells and create space for the transplanted cells, don’t need to do with SCID patients because they are already devoid of T cells, busulfan, cyclophosphamide, anti thyocyte globulin), immunosyppressive treatment with cyclosporin A, in this case all myeloid cells in the patient were shown to be of donor origin (full chimerism) 28 days after tranplant and all of the expressed normal amount of CD18

**Describe how and where lymphocytes are formed and how they migrate and recirculate in the body.**

RBCs, monocytes, granulocytes, and B lymphocytes are formed and differentiate in the bone marrow whil T cells differentiate from the thymus. Leukocytes (WBCs) enter the blood stream and emigrate from the blood to perform their effector functions. Lymphocytes recriculate through secondary lymphoid tissues where they are detained if they encounter an antigen to which they can respond, macrophages migrate into tissues as they mature from circulating monocytes, effector T cells and large numbers of granulocytes are recruited to extravascular sites in response to infection or injury.

**Describe markers of T lymphocytes (CD3, CD4 and CD8), B cells (CD19), and NK cells (CD16).**

LAD patents show decreased CD18 expression on their CD3 T cells

**Explain that white blood cells (leukocytes) in contrast to red cells perform the majority of their effect functions in extravascular sites.**

RBCs spend their entire lifespan (120 days) in the bloodstream. WBCs emigrate from the blood to perform their function at specific sites.

Describe the steps involved in migration of leukocytes from blood into tissues that is seen in response to situations such as infection. Explain that these involve a change from a **rolling interaction** (where there is weak temporary attachment to endothelial E-selectin), to **tight binding** (involving interaction of leukocyte integrins with induced ICAM-1 and with chemokine molecules already on the endothelial surface). After this has occurred, the leukocyte integrins further contribute to **diapedesis** (passage through the junctions between endothelial cells and into the underlying tissue). Then leukocytes move towards sites of infection by following gradients of chemokines. (Fig 3.1)

Leukocyte flow is slowed by reversible interactions between selectins on the surface of activated vascular endothelium and fuxosylated glycoproteins on the leukocyte surface (ex: sialyl Lewis) leading to rolling on the endothelium (making and breaking contact). This allows tight binding of the leukocytes to the endothelial surface using ICAM1 on the endothelium surface, triggered by actions of chemokines retained on heparan sulfate proteoglycans on the endothelial surface (ex: CXCL8 recognized by the CXCR1 receptor on leukocytes), which activate the leukocyte integrins (LFA1 and Mac1) to adhere more tightly to their receptors. Tight binding arrests the rolling and allows the leukocyte to extravasate (leave the bloodstream) by squeezing between the endothelial cells forming the wall of the blood vessel via diapedesis (leukocyte integreins are required from this process and migration toward chemoattractants). Adhesion between molecules of CD31 expressed on the leukocyte and the junction of the endothelial cells also contributes to diapedesis. Leukocyte then migrates along a concentration gradient of chemokines secreted by the cells at the site of infection.

**Explain that lymphocytes entering secondary lymphoid tissues undergo diapedesis (e.g. at the high endothelial venule) but the initial step of rolling adhesion involves the interaction of T cell associated L-selectin with mucin-like addressins.**

L selectin is expressed on naïve T cells and binds to vascular addressins CD34 (expressed on endothelial cells but not exclusive to HEVs) and GlyCAM1 (exclusively on HEVs) on high endothelial venules to enter lymph nodes. Addressin MAdCAM1 is expressed on mucosal endothelium and guides entry of naïve T cells into mucosal lymphoid tissue. L selectin recognizes carb moieties on the vascular addressins.

**Explain how the Rebuck skin window test is performed and what it measures**

Skin of the forearm is abraded with a scalpel and a coverslip is placed on the abrasion. After 2 hrs. the coverslip is removed and replaced by another coverslip every 2 hrs for a total of 8 hrs. The migration of immune cells into the damaged skin is monitored.

In normal patients: first coverslip shows neutrophils, monocytes appear at 4 hrs, predominately monocytes at 8 hrs.

In LAD patients, no white cells accumulate on the coverslips because they are unable to emigrate from the bloodstream and onto the coverslip (show high levels of WBCs in the blood stream, characteristic finding).

**Describe that inability to make CD18 (a component of 2 integrins) leads to lack of recruitment of white cells into tissues (leaving high white cell numbers in the blood) and to susceptibility to infection. Explain that cells do not accumulate on the coverslip of a Rebuck glass window in this setting and that the condition results in leukocyte adhesion deficiency.**

**Describe that persons with leukocyte adhesion deficiency, and who survive, develop severe gingivitis, and have very poor wound healing.**

**Explain that the healing deficiency in leukocyte adhesion deficiency is associated with a greatly delayed separation of the umbilical cord.**

**Case 28: Recurrent herpes simplex encephalitis (HSE)**

**Summary:** IFNa and b provide ubiquitous cell mediated antiviral defense. Induction of IFNs is transcriptionally regulated and it elicited by a variety of upstream receptors. TLR3, 7, 8, and 9 recognize viral components and are located in endosomal membranes and bind nucleic acid intermediates generated during intracellular viral replication. TLR3 binds dsRNA, TLR7 and 8 bind ssRNA, and TLR9 binds dsDNA. All the TLRs require association with the endosomal ER membrane protein UNC93B for signaling but differ in their pathway components that are downstream of the receptors. TLR7, 8, and 9 use MyD88 and ser/threonine kinases IRAK4 and IRAK1 to activate the IKKa:b:g proteine complex, leading to activation of NFkB transcription factor. Also induces IFN regulatory factor IRF7 by an alternative signaling pathway that involves MyD88 and IRAK4 independent of IKK. TLR3 signals through the adaptor protein TRIF to activate a pathway using protein kinases TBK1 and IKKe leading to IRF3 and 7 activation. IRF3 and 7, and NFkB can activate type 1 IFN genes (links the activation of TLRs recognizing different viral replication intermediates to the production of type 1 IFNs).

After IFNa and b are produced and released by the virus infected cell, they bind to their common receptor (heterodimer of IFNaR1 and IFNaR2) on the surface. Results in activation of JAK1 and TYK2 kinases and of the TF ISGF3 (heterotrimeric complex composed of STAT1 and 2 and IRF9). ISGF3 binds to IFN stimulated response element (ISRE) within the promoter of type 1 IFN dependent genes to induce their transcription and trigger antiviral activity leading to destruction of the virus.

HSV1 is a dsDNA virus, that is very common in the population (85% of adults have antibodies) and is typically associated with infections of the oral mucosa (oral ulcers) or of the eye (conjuncitvitis and keratitis). After replication at initial infection site, the virus is transported through sensory neurons to the trigeminal nerves and ganglia where it establishes a latent infection. Reactivation occurs as herpes labialis (cold sores) in about 30% of the infected population. Although infection is usually benign, on rare occasions it can invade the brain through the olfacotry tract and trigeminal nerves and infect both neuronal and glial cells, causing herpes simplex necrotizing encephalitis. Temporal and parietal lobes are typical targets.

Defects along the TLR3 signaling pathway are associated with increased susceptibility to recurrent HSE. The dsRNA intermediate generated during HSV1 replication is recognized by endosomal TLR3. Genetic defects in TLR3, UNC93B, and TRAF3 have been identified among patients with HSE. Patients with NEMO (IKKg) deficiency show increased susceptibility to HSE along with more complex clinical and immunological phenotype, including increased susceptibility to mycobacterial disease. The NEMO deficient patients don’t show disseminated HSV1 disease nor increased susceptibility to other viral infections, suggesting that HSE results from inability of CNS cells to respond to HSV1 through TLR3 dependent mechanisms.

HSE can also result from genetic defects in the TF STAT1. In addition to activation by type 1 IFNs, STAT1 can be activated via different receptors that respond to IFNg (doesn’t have antiviral activity but promotes Th1 activation of macrophages). Receptor activation leads to formation of gamma activated factor (GAF, heterodimer of STAT1) that promotes transcription of IFNg genes. 3 forms of STAT1 deficiency: autosomal recessive (null mutation of STAT1 gene resulting in increased susceptibility to severe viral disease including HSE and mycobacterial due to failure of macrophage activation, formation of both ISGF3 and GAF are impaired), dominant negative heterozygous (increase susceptibility to mycobacterial disease but not severe viral infections, ISGF3 formation is still possible so type 1 IFN dependent anti viral responses aren’t imparied), and heterozygous gain of function mutations (chronic mucocutaneous candidiasis).

Patients with MyD88 or IRAK4 deficiency suffer from recurrent infections with pyogenic bacteria but don’t show increased susceptibility to HSE or other viral infections even if their fibroblasts didn’t produce type 1 IFNs in response to stimulation of TLRs 7-9. Indciates that these IRAK4 dependent TLR-mediated IFN responses are redundant for protective immunity to viruses such as HSV1.

**Case:** 6 mo old girl with parents from French Guiana, high fever, vomiting and R hemiclonic seizures (treated with diazepam), EEG shows spike waves in L temporal lobe, MRI shows hyperintensity of signal in L temporal lobe, CSF suggested viral meningoencephalitis (mostly lymphocytes and tested positive for HSV1 Ag), confirmed by PCR, increased WBCs, lymphocytosis, normal IgG, IgA, and IgM, treated with IV acyclovir, able to mount Ab response, at 2 years had common viral infections of early childhood, at 3 yrs again developed high fever, R sided tonico clonic seizures, paralysis of R face and arm (bronchio facial paralysis) and photophobia, CSF shows recurrence of meningoencephalitis with lymphocytes, MRI reveled new lesion in L parietal lobe and L thalamus, genetic testing showed homozygous single NT deletion in UNC93B exon, no other viral episodes but still has paralysis or R arm and face

**Explain the role of type I interferons in controlling viral infections.**

**Describe the pathogenesis of recurrent herpes simplex encephalitis (HSE).**

**Explain why the genetic defects leading to recurrent herpes simplex encephalitis (HSE).**

**Explain why patients with TLR-3 signaling degects present increased susceptibility to recurrent herpes simplex encephalitis (HSE).**

TLR3 signals through the adaptor protein TRIF to activate a pathway using protein kinases TBK1 and IKKe leading to IRF3 and 7 activation. IRF3 and 7, and NFkB can activate type 1 IFN genes (links the activation of TLRs recognizing different viral replication intermediates to the production of type 1 IFNs). Defects along the TLR3 signaling pathway are associated with increased susceptibility to recurrent HSE. The dsRNA intermediate generated during HSV1 replication is recognized by endosomal TLR3.

**Case 29: Interleukin 1 Receptor-associated Kinase-4 (IRAK4) Deficiency**

**Case:** 6 yr old boy with reucrrent pyogenic infections (pneumococcal meningitis, frequent otitis media), intesitnal intussusceptions (telescoping of intestinal segment into adhacent segnent) at 11 mo complicated by perforation of intestina and formation of abscess in periotneum, suffered from boils/furuncls of the scalp and several skin infections that responded to treatment, weak febrile response that only occurred late in infections, no particular susceptibility to viral infections, thin, enlrged tonsils and anterial cervical lymph nodes (lymphadenopthay), enlarged liver, normal complete blood county, complement function, and Ig titer, have protective immune responses to protein Ag (tetanus toxoid) but not to polysaccharide Ags (pneumococcus or meninococcus), normal T and B cell numbers, DNA sequencing showed homozygous nonsense mutation within kinase domain of IRAK4 gene that produced a truncated protein with no kinase activity

**Treatment:** maintained in good health into adolescence by combo of prophylactic antibiotics and regular infusions of IV Ig

TLR function evaluated: sample of peripheral blood cells stimulated by ligands to TLRs to see if there was TNFa response (use phorbol myristate acetate plus ionomycin as positive control because they bypass receptor signaling by activating PKC and cause an influx of Ca), IRAK4 deficient patients would show little or no TNFa production, also tested to see if signal transuction pathway worked by testing cells responses to IL1 (since the IL1R uses the same signaling pathway), stimulation with IL1 produced no activation of MPA kinases and no phosphorylation of IkBa, consistent with defect very early in the pathway (IL1R associated kinase 4),

**Recognize pathogen-associated molecular patterns (PAMPs) interact with invariant pattern recognition receptors (PRRs) that are found on many cell types, including dendritic cells, neutrophils and macrophages.**

Presence of invading microbes is first recognized by tissue macrophages at the site of infection. Pathogens carry characteristic chemical structures on their surface and in their nucleic acids that aren’t present in human cells (PAMPs). These are recognized by invarient PRRs on macrophages and neutrohpils which take up the pathogen and destory it, as well as DCs which present Ag and activate naïve T cells (link between innative and adaptive immune response). TLRs are PRRs that are present on the cell surface and in the membranes of endosomes to enable cells of the innate immune system to detect and respond to a wide variety of pathogens.

10 different TLRs that recongize microbe Nas (unmethylated bacterial DNA and long dsRNA) and molecules specific to classes of microbes (LPS/endosomes on gram neg bacteria, flagellin protein on flagella). Molecules are essential for the microbes survival and are therefore invarient. Therefore a limited number of TLRs can detect the presence of many different pathogens by recognizing features that are typical of that type of pathogen.

**List Toll-like receptor (TLRs) as being types of PRRs and that some of their most important activities include activation of NF-B and upregulation of proinflammatory cytokines, such as TNF.**

Signaling receptors. Activation lead to enhancement of antimicrobial activity of macrophages and neutrophils and the secretion of cytokines by macrophages which help attract more macrophages and neutrophils out of the blood and into the site of infection. Upon engagment of TLRs, DCs undergo a brief period of macropinocytosis that is important for taking up and processing microbial antigens for presentation to naïve T cells. Signaling also induces maturation of DCs and their migration to peripheral lymphoid tissues such as the lymph nodes where they can encounter and activate Ag specific T cells. During maturation, DCs upregulate the production of costimulatory molecules (CD40, B7) which are essential for T cell activation and production of chemokines and cytokines that help induce adaptive immune responses (TNFa, IL1, IL6, and IL12). Plasmacytoid DCs produce large amounts of antiviral type 1 IFNs (IFNa and b) in response to viral infections.

Stimulation of DCs by PAMPs such as LPS and unmetylated CpG in bacterial DNA is critical for their ability to induce development of Th1 effector cells. Exposure of DCs to produce pro inflammatory cytokines is sufficient to enable them to induce proliferation (clonal expansion) of Ag specific naïve T cells, but the upregulation of costim molecules and IL12 producction as results of TLR stimulation is essential for DCs to be able to induce differentiation of Th1 effector functions such as secretion of IFNg in CD4 T cells. Thus TLRs have a vital role in both innate and adaptive immune responses.

**Describe the activation of cytokine production, in that they often follow PRR (e.g. TLR) engagement with ligands and involve several signaling pathways leading to activation of genes under control of transcription factors NF-B, as well as IRF3 and IRF7. (Fig 8.1)**

TLR 3 (rec dsRNA) and TLR4 (rec LPS) activation IRF3 and production of type 1 IFN

TLR1-5, 7-9 activate NFkB for production of TNFa and IL6

TLR7-9 activation IFN7 for production of type 1 IFN

TLR1 and 2 recognize PG, LP and GPI, TLR5 rec flaggelin, TLR 9 rec CpG

TLR 7-9 are on endosomal membranes

**Recognize that the IL-1 receptor (IL-1R) and TLR signaling pathways share MyD88, which interacts with IRAK4 protein kinase. IRAK4 acts early in the pathway for both IL-1R and TLRs.**

IL1R and the TLRs share a common signal transduction pathway that involves adaptor protein MyD88, signaling intermediate TRAF6, and receptor associated protein kinases IRAK1 and IRAK4. Activation of receptors leads to recruitment of MyD88 to the receptor followed by recruitment and activation of IRAK 4 (the initial protein kinase). This is followed by recruitment of IRAK1 and TRAF6 to the pathway. Pathway can then diverge with 1 branch activating the MAP kinases and the other activating NFkB. Activation of the classical NFkB pathway results in production of proinflammatory cytokines IL6, IL12, and TNFa. Functional IRAK4 is critical to the ability to make responses to TLR ligands because signaling via NFkB is blocked in its absence.

Exceptions because TLR3 and 4 can also signal via a pathway that doesn’t use IRAK4. Can use a second MyD88 independent pathway involving adaptor proteins TRIF (for TLR3) and TRIG/TRAM (for TLR4), which results in activation of IFN regulatory factor 3 (IRF3) and the production of type 1 IFNs (essential for function of innate response to viruses). Type 1 IFN production also stimulated in plasmacytoid DCs and monocytes by activation of TLR7-9 which can signal via the IRAK/TRAF6 pathway leading to production of proinflammatory cytokines. However, IRAK1 can phosphorylate and activate IRF7 TF which induces expression of genes encoding IFNa. Virus induced activation of IRF7 and subsequen IFNa production is essential for host defense against viruses.

IRAK 4 may also be directly involved in development of optimal adaptive immune responses. Ag stimulated T cells from mice with inactivated IRAK4 kinase secrete reduced amounts of IL18 that is important in antibacterial immunity. RIAK4 lacking mice also have reduced splenic and peripheral expansion of CD8 T cells in repsonse to infection with lymphocytic choriomeningitis which suggests tht IRAk4 may be requires for optimal antiviral CD8T cell activity.

**Explain that loss of IRAK4 activity causes an immunodeficiency which is characterized by an impaired antibody response to polysaccharide antigens, resulting in a tendency to have recurrent *Neisseria meningitidis* and *Streptococcus pneumoniae* infections. Both of these bacteria have antiphagocytic polysaccharide capsules and normal protection against them requires developing opsonizing IgG antibodies.**

IRAK4 deficient patients seem to be primarily suseptible to pyogenic bacteria (severe, recurrent infections). Susceptibility to invasive bacterial infections decreases with age (possibly due to compensation by adaptive immune system).

Fail to mount antibody response to polysaccharide antigens because the initial signaling in DCs seems to require TLR2 which recognizes lipoteichoic acid of G positive bacterial cell walls and lipoportines of gram negative because, as well as TLR4 which recognized LPS. May be because stimulation of DCs via these TLRs is essential for their ability to induce differentiation of T cells (esp Th1) to help B cells make antipolysaccharide antibodies. Reponse to pneumovax for example seems to depend on the presence of such TLR ligands in the vaccine and has been shown that depltiton of endotoxin form the vaccine renders it unable to elicit antibody response in mice.

See lack of fever because in IRAK4 deficiency there is a early block in the TLR/IL1R signaling pathway so little if any pro inflammatory cytokines are produced and there is an impaired febrile response. \*History of little or no fever associated with recurrent pyogenic infections supports diagnosis of IRAK4 deficiency

Little increased susceptibility to viral infections. Surprising because production of type 1 IFNs in response to ligation of TLR 7-9 is diminished in IRAK4 patients and IFN synthesis is variably affected in response to TLR3 ligation with dsRNA. One explanation could be that humans can make relatively intact adaptive immune response to viruses as result of responses by Tc cells and antiviral Abs produced by B cells. Probably redundancy between adaptive and innate responses responses with innate NK cells cytotoxic for virus infected cells being activated by T cell derived IL2 and IFNg. Other intracellular antiviral immune responses due to activation of RNA dependent protein kinase pathway and activation of cytoplasmic RIG1 protein by dsRNA leading to synthesis of type 1 IFNs.

**Explain that persons with IRAK4 deficiency usually have normal numbers of T and B cells.**

Also normal serum Ig levels, protective Ab titers to protein Ag but variably impaired AB titers to polysaccharide Ags.

**Other conditions that have increased susceptibility to pneumococcal infections:**

Defects of innate immunity including congenital asplenia and defects of complement pathways, defects in NFkB pathway downstream of TLRs and surface receptors include NEMO deficiency and mutations in IkB that prevent its degradation and release of NFkB

Defects of adaptive immunity resulting in impaired antibody production such as X linked agammaglobulinemia and SCID result in increased susceptibility to gram positive bacteria such as pneumococci and staphylococci

(Note mistake in text, page 47, 7 lines from base.

Meningococcus is the general name given to *Neisseria meningitidis*; there is no *Streptococcus meningitidis.*)